

SEQ ID NO:1:

GGCATATTAGCTGGGTTACTGTGAATTTCTGACAGGTCAGCTGCATGCCACAGA  
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 GCCCCAGTTGAGAAAACCTCTGGAACAAACTCCTAGGGGCTACCTCACTTCCTTGA  
 GATGTTAACAGCACTTATAAGCTCTACACATAGTTACCTGGGATTGGATTGAA  
 AGCTGCAAGACTAGCAACCTGGAGGCCCTGGAGACAGAAGGGACTGATGGGCACA  
 CTTCCGGAGTGCCTGT

SEQ ID NO:2:

GCGGGCTGCCCGCAAGGGTGGCGCGCGCGTCTTCTTGTCTGGTCAACAAAG  
 AAATGTGGAGTGTCTTGGCTGAATCCTCATACAGACAAGATCATTATGGTGCTGTTA  
 GGTTGAAAAAGTGATATAATAAGGAACCAAGGAGAAAATTAGAAGGAAAGAAA  
 AAATTGCCTCTGCAGGTGTGCGAGCAGGATTGCTTCTGCAACAAAAGCCTCCACCCA  
 GCCACATCTTGGGAAAAGAATGCCACTTCTTGGGCACAGTCTTTTATGCTGGT  
 GGTATCCTGTGTTGCAGCGCTGTCTCCCACAGGAACCAGCAGACTTGGTTGAGGG  
 TATCTCCTGTCTTCCATGTGCCCATCAATGTCAGCGCCAGCACCTGTATGGAATT  
 ATGTTGATGCAGGGAGCACTGGAACTCGAATTGTTACACCTTGTGCAGAAA  
 ATGCCAGGACAGCTCCAATTCTAGAAGGGGAAGTTTGATTCTGTGAAGCCAGGA  
 CTTCCTGCTTGTAGATCAACCTAACGAGGGTGCTGAGACCGTTCAAGGGCTCTA  
 GAGGTGGCCAAAGACTCAATCCCCGAAGTCAGTGGAAAAAGACCCAGTGGCCT  
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 CAGGTCTGCTGCATGGCCACAGACAGGAGACTGTGGGACCTGGACCTAGGGGGA  
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 GACAGAAGGGACTGATGGGACACTTCCGGAGTGCCTGTTACCGAGATGGTTGG  
 AAGCAGAGTGGATCTTGGGGTGTGAAATACCACTGTTGGCAACCAAGAAGGG  
 GAGGTGGGCTTGGAGCCCTGCTATGCCAGTGTGCTGAGGGTGGTACGAGGAAA  
 ACTTCACCAAGCCAGAGGAGGTCCAGAGAGGTTCTATGCTTCTTACTATTATGA  
 CCGAGCTGTTGACACAGACATGATTGATTGAAAGGGGGTATTAAAAGTTGA  
 AGATTGAAAGAAAAGCCAGGGAAAGTGTGTGATAACTGGAAAACCTCACCTCAG  
 GCAGTCCTTCTGTGCATGGATCTCAGCTACATCACAGCCCTGTTAAAGGATGGCT  
 TTGGCTTGCAGACAGCACAGTCTTACAGCTCACAAAGAAAAGTGAACAAACATAGAG  
 ACGGGCTGGGCCTGGGGCACCTTACCTGTTGCAACACCTTTAAGGGGAGGA  
 TGAGGCCACGTACTTCTGGAGACCTGCATTGCCAACACCTTTAAGGGGAGGA  
 GAGAGCACTTAGTTCTGAAGTAGCTGGGACATCCTGGACTTGAGCCTAGAGATT  
 WRGTTAATTAAASCAGGCCAGCTTATCCTTWATRAGGTAATTACTGCMGGCG  
 TTTACACGTCGTGATGGNAAACCTGCGTCCAACTAACGCTTGA  
 SAMATCCCCTCG  
 CAGCTGCGATAACCAAGCCGACGACGCCCTCACAGTGCCA

Figure 1

SEQ ID NO:3.

MATSWGTVFMLVVSCVCSAVSHRNQQTWFEGIFLSSMCPIVSASTLYGIMFDAGSTG  
TRIHVYTFVQKMPGQLPILEGEVFDSVKPGLSAFVDQPKQGAETVQGLLEVAKDSIPRSH  
WKKTPVVLKATAGLRLLPEHKAKALLFEVKEIFRKSPFLVPKGSVSIMDGSDEGILA WV  
TVNFLTGQLHGHQRQETVGTLDLGGASTQITFLPQFEKTL\_EQTPRGYLT SFEMFNSTYKL Y  
THSYLGFGLKAARLATLGALETEGTDGHTFRSACLPRWLEAEWIFGGVKYQYGGNQEG  
EVGFEP CYAEVLRVVRGKLHQPEEVQRGSFYAFSYYYDRAV DTD MIDYEKGGILKVED  
FERKAREVCDNLENFTSGSPFLCMDLSYITALLKDGF GFADSTV LQLTKKVNNIETGWA  
LGATFHLLQSLGISH

Figure 2

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Figure 3

1 IMATSWGTUVFFMLVVSCVICS~~SAVSHRNQCTWFEQIF~~SSMCP 246 prot  
1 MATSWGAVF-IMLAAACVGSCTV~~YIEFQCTWFEQVFLSSMCP~~ mut ncpase

41 ENVSAS~~T~~Y~~G~~IMFDAGSTGTRI~~H~~VYTFV~~C~~AM~~E~~GC~~I~~FILEG 246 prot  
40 ENVSACT~~F~~Y~~G~~IMFDAGSTGTRI~~H~~VYTFV~~C~~AM~~E~~GC~~I~~FILEG mut ncpase

81 EVFDSVKPGLSAFV~~D~~QPKQGAETVQ~~G~~ILEVAKDSI~~F~~R~~S~~HW 246 prot  
80 E~~V~~FDSVKPGLSAFV~~D~~QPKQGAETVQ~~G~~ILEVAKDSI~~F~~R~~S~~HW mut ncpase

121 KIK~~T~~PVVVKATAGLRLP~~E~~H~~I~~K~~A~~K~~L~~L~~E~~IF~~E~~VI~~K~~E~~I~~FR~~K~~IS~~F~~FLV~~P~~ 246 prot  
120 E~~T~~ATPVVVKATAGLRLP~~E~~H~~I~~K~~A~~K~~L~~L~~E~~VE~~E~~VI~~K~~N~~S~~PF~~L~~V~~P~~ mut ncpase

161 KIGSVSIMDGSD~~E~~G~~I~~LA~~W~~V~~T~~V~~N~~FL~~T~~G~~Q~~L~~H~~G~~H~~R~~Q~~E~~T~~V~~G~~T~~I~~D~~D~~ 246 prot  
160 KIGSVSIMDGSD~~E~~G~~I~~LA~~W~~V~~T~~V~~N~~FL~~T~~G~~Q~~L~~H~~G~~H~~R~~Q~~E~~T~~V~~G~~T~~I~~D~~D~~ mut ncpase

201 G~~G~~ASTQ~~I~~TF~~L~~P~~Q~~FE~~K~~T~~L~~E~~Q~~T~~P~~RG~~Y~~LT~~S~~F~~E~~M~~F~~N~~S~~T~~Y~~K~~L~~Y~~T~~H 246 prot  
200 G~~G~~ASTQ~~I~~TF~~L~~P~~Q~~FE~~K~~T~~L~~E~~Q~~T~~P~~RG~~Y~~LT~~S~~F~~E~~M~~F~~N~~S~~T~~Y~~K~~L~~Y~~T~~H mut ncpase

241 S~~Y~~LG~~F~~GLKAARL~~A~~L~~T~~GA~~E~~T~~E~~G~~T~~D~~G~~H~~T~~F~~R~~S~~A~~C~~L~~P~~R~~W~~L~~E~~A~~E 246 prot  
240 S~~Y~~LG~~F~~GLKAARL~~A~~L~~T~~GA~~E~~AK~~G~~T~~D~~G~~H~~T~~F~~R~~S~~A~~C~~L~~P~~R~~W~~L~~E~~A~~E~~ mut ncpase

281 W~~I~~F~~G~~GV~~K~~YQ~~Y~~GG~~N~~Q~~E~~G~~E~~V~~G~~F~~E~~PC~~Y~~A~~E~~V~~L~~R~~V~~V~~R~~G~~K~~L~~H~~Q~~P~~E~~E~~ 246 prot  
280 W~~I~~F~~G~~GV~~K~~YQ~~Y~~GG~~N~~Q~~E~~G~~E~~M~~G~~F~~E~~PC~~Y~~A~~E~~V~~L~~R~~V~~V~~Q~~G~~K~~L~~H~~Q~~P~~E~~E~~ mut ncpase

321 V~~Q~~R~~G~~S~~F~~Y~~A~~F~~S~~Y~~Y~~D~~R~~A~~V~~I~~D~~T~~D~~~~M~~I~~D~~Y~~E~~K~~G~~G~~I~~L~~K~~V~~E~~D~~F~~E~~R~~K~~A~~R 246 prot  
320 V~~Q~~R~~G~~S~~F~~Y~~A~~F~~S~~Y~~Y~~D~~R~~A~~A~~D~~D~~H~~I~~I~~D~~Y~~E~~K~~G~~G~~V~~L~~K~~V~~E~~D~~F~~E~~R~~K~~A~~R mut ncpase

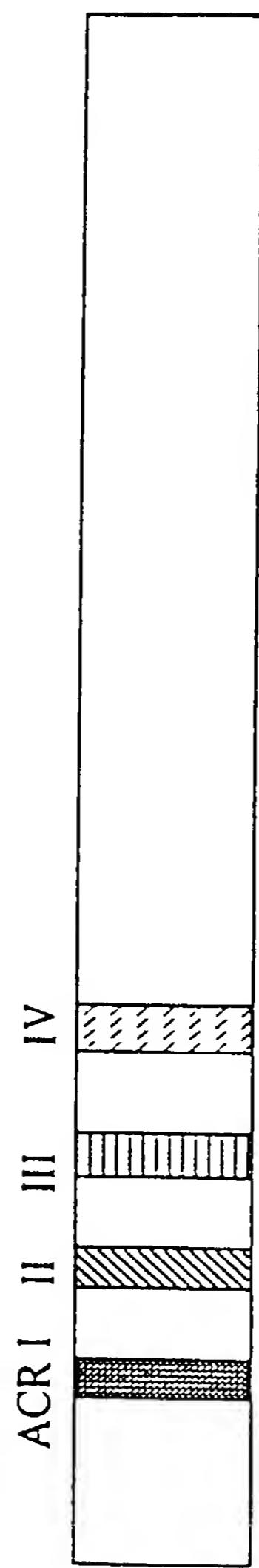
361 E~~V~~C~~D~~N~~L~~E~~I~~N~~F~~T~~S~~G~~S~~P~~F~~L~~C~~M~~D~~L~~S~~Y~~I~~T~~A~~L~~L~~K~~D~~G~~F~~G~~F~~A~~D~~S~~T~~V~~L~~Q 246 prot  
360 E~~V~~C~~D~~N~~L~~G~~S~~F~~S~~S~~G~~S~~P~~F~~L~~C~~M~~D~~L~~T~~X~~I~~T~~A~~L~~L~~K~~D~~G~~L~~G~~F~~A~~E~~R~~H~~P~~L~~T~~ mut ncpase

401 E~~T~~K~~K~~V~~N~~N~~I~~E~~T~~G~~W~~-A~~L~~G~~A~~~~F~~-----H~~L~~L~~O~~S~~L~~G~~I~~S~~H~~ 246 prot  
400 -E~~A~~H~~K~~E~~S~~E~~Q~~H~~R~~D~~W~~L~~G~~G~~G~~H~~S~~P~~A~~P~~V~~S~~G~~H~~W~~Q~~U~~R~~P~~S~~S~~T~~S~~E~~A~~C~~A~~ mut ncpase

428  
439 L~~S~~E~~P~~V~~F~~S~~Q~~E~~G~~V~~D~~S~~E~~T~~F~~S~~D~~L~~S~~G~~K~~A~~W~~P~~E~~T~~R~~ 246 prot  
438 L~~S~~E~~P~~V~~F~~S~~Q~~E~~G~~V~~D~~S~~E~~T~~F~~S~~D~~L~~S~~G~~K~~A~~W~~P~~E~~T~~R~~ mut ncpase

Figure 4

## Apyrase Conserved Regions in CD39-L4



ACR I    CD39-L4    DAGST    G    T    R    I  
 CD39-L4    mutant    DAGST    H    T    S    I  
 CD39    DAGSS    H    T    S    L

ACR II    CD39-L4    PV    V    L    K    ATAGLRLLL  
 CD39-L4    mut    PV    Y    L    G    ATAGLRLLL  
 CD39    PV    Y    L    G    ATAGMRLLL

ACR III    CD39-L4    M    D    T    S    DEGI    L    AWVTVN  
 CD39-L4    mut    M    T    E    Q    DEGI    F    AWVTVN  
 CD39    EEGA    Q    Q    GWITIN

FIG. 5

Nucleotide sequence of the CD39-L4 mutant ACR III (SEQ ID NO:6). The nucleotide changes have been highlighted. The G to A and A to C changes at positions 502 and 503 produce a Thr, the T to C, C to A and C to A changes at positions 508-510 result in a Gln and the A to C changed at position 525 result in a Phe.

```

ATGGCCACTTCTGGGGCACAGTCTTTCATGCTGGTGGTATCCTGTGTTGCAGCGCTGTCT
CCACAGGAACCAGCAGACTTGGTTGAGGGTATCTCCTGTCTCCATGTGCCCATCAATGT
CAGCGCCAGCACCTGTATGGAATTATGTTGATGCAGGGAGCACTGGAACTCGAATTCATGTT
TACACCTTGTGCAGAAAATGCCAGGACAGCTCCAATTCTAGAAGGGGAAGTTTGATTCTG
TGAAGCCAGGACTTTCTGCTTTGATCAACCTAACGAGGGTGTGAGACCGTTCAAGGGCT
CTTAGAGGTGGCCAAAGACTCAATCCCCGAAGTCACTGGAAAAGACCCAGTGGCCTAAAG
GCAACAGCAGGACTACGCTACTGCCAGAACACAAAGCCAAGGCTTGCTCTTGAGGTAAAGG
AGATCTTCAGGAAGTCACCTTCTGGTACCAAAGGGCAGTGTAGCATCATGACTGGACAA
CGAAGGCATATTCGTGGTTACTGTGAATTTCTGACAGGTAGCTGCATGGCCACAGACAG
GAGACTGTGGGACCTGGACCTAGGGGAGCCTCACCCAAATCACGTTCTGCCAGTTG
AGAAAACTCTGGAAACAAACTCCTAGGGCTACCTCACTCCTGTGAGATGTTAACAGCACTTA
TAAGCTCTACACATAGTACCTGGGATTGGATTGAAAGCTGCAAGACTAGCAACCCTGGGA
GCCCTGGAGACAGAACGGACTGATGGGCACACTTCCGGAGTGCCTGTTACCGAGATGGTTGG
AAGCAGAGTGGATCTTGGGGGTGTGAAATACCAGTATGGTGGCAACCAAGAACGGGAGGTGGG
CTTGAGGCCCTGCTATGCCGAAGTGCTGAGGGTGGTACGAGGAAAACTCACCAGGCCAGAGGAG
GTCCAGAGAGGTTCTATGCTTCTACTATTATGACCGAGCTGTTGACACAGACATGA
TTGATTATGAAAAGGGGGTATTTAAAAGTTGAAGATTGAAAGAAAAGCCAGGGAAAGTGTG
TGATAACTTGGAAAACTTCACC TCAGGCAGTCCTTGTGCATGGATCTCAGCTACATCAC
AGCCCTGTTA AAGGATGGCTTGGCTTGAGACAGCACAGTCTACAGCTCACAAAGAAAGT
GAAC AACATAG AGACGGGCTGGGCCTGGGGCACCTTCACCTGTTGCAGTCTCTGGCA
TCTCCCATTGA

```

Amino acid sequence of CD39-L4 mutant ACR III (SEQ ID NO:7). The amino acid changes are D to T (a.a. 168), S to Q (a.a. 170) and L to F (a.a. 175). The changes are shown in bold.

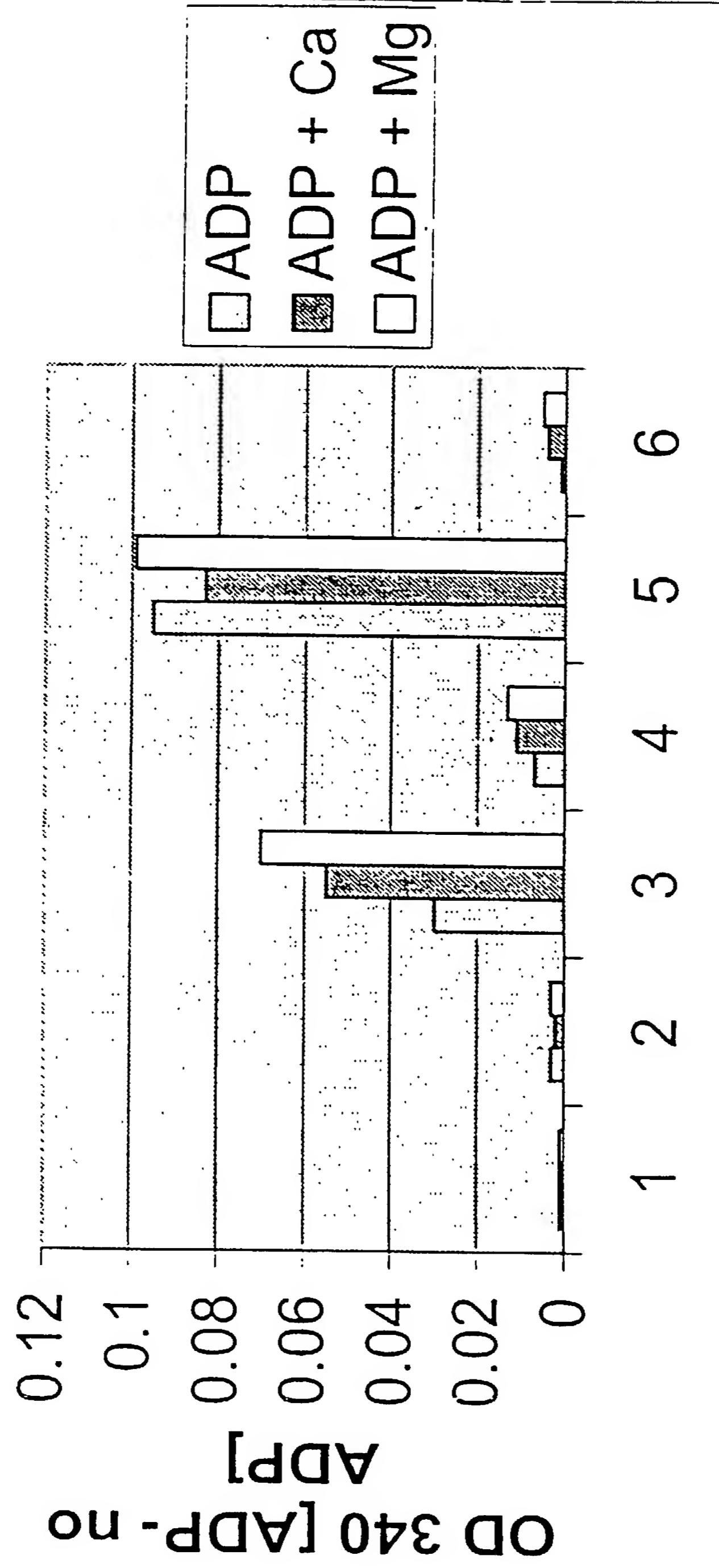
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MATSYGTVFMLVVSCVCSAVSHRNQQTWFEGIFLSSMCPINVASTLYGIMFDAGSTGT
RIHVYTFVQKMPGQLPILEGEVFDSVKPGLSAFVDQPKQGAETVQGLLEVAKDSIPRSHW
KKTPVVLKATAGLRLPEHAKALLFEVKEIFRKSPFLVPKGSVSIMTGQDEGIFAWVTV
NFLTGQLHGHHRQETVGTLDLGGASTQITFLPQFEKTLEQTPRGYLTSEMFNSTYKLYTH
SYLGFGLKAARLATLGAETEGTDGHTFRSACLPRWLEAEWIFGGVKYQYGGNQEGEVGF
EPCYAEVLRVVRGKLHQPEEVQRGSFYAFSYYYDRAVDTDMIDYEKGGILKVEDFERKAR
EVCDNLENFTSGSPFLCMDLSYITALLKDGFGFADSTVLQLTKVNNIETGWALGATFHL
LQSLGISH

```

FIG. 6

## FIG. 2 ADPase activity of CD39-L4 ACR mutants



1) ACR I; 2) ACR II; 3) ACR III; 4)  
Wild Type; 5) CD39; 6) Vector

FIG. 7

SEQ ID No-3	MAT SWG	- - - - -	V F F M L V V S C V C S A V S H R N Q Q T W F E G I I L S S M C P I N V S A S	46
SEQ ID No-5	MAT SWGT	- - - - -	V F F M L V V S C V C S A V S H R N Q Q T W F E G I I L S S M C P I N V S A S	46
CD39Human.seq	MEDTKESNVKTFC	S K N I L A I L G F S S I I A V I A	- - - - -	LLAVGLTQ - - N K A L P E N 46
SEQ ID No-3	TLYGIMFDAG	S T G T R I H V Y Y T F V Q K M P G Q L P I L E G E V F D S V K	- P G L S A F V D Q P K Q G	100
SEQ ID No-5	TLYGIMFDAG	S T G T R I H V Y Y T F V Q K M P G Q L P I L E G E V F D S V K	- P G L S A F V D Q P K Q G	100
CD39Human.seq	V K Y G I V L D A G S S H I T S L Y I Y	K W P A E K E N D T G V V H Q V V E C R V K G P G I S K F V Q K V N E I	- - - - -	101
SEQ ID No-3	A E T V Q G I L E V A K D S I P R S H W K K T P V V L K A T A G L R L L	- - - - -	- P E H K A K A L L F E V K E I F	152
SEQ ID No-5	A E T V Q G I L E V A K D S I P R S H W K K T P V V L K A T A G L R L L	- - - - -	- P E H K A K A L L F E V K E I F	152
CD39Human.seq	G I Y L T D C M E R A R E V I P R S Q H Q E T P V Y L G A T A G M R L L	R M E S E E L A D R V I L D V Y E R S L	- - - - -	156
SEQ ID No-3	R K S P F L V P K G S V S I M D G S D E G I L A W V T V N F L T G Q L	- - - - -	- H G H R Q E T V	195
SEQ ID No-5	R K S P F L V P K G S V S I M D G S D E G I L A W V T V N F L T G Q L	- - - - -	- H G H R Q E T V	195
CD39Human.seq	S N Y P E D F Q - - G A R I I T G Q E E G A Y G W I T I N Y L I G K F S Q K T R W F S I V P Y E T N N Q E T F	R M E S E E L A D R V I L D V Y E R S L	- - - - -	209
SEQ ID No-3	G T L D L G G A S T Q I T F L P Q F E K T L E Q T P R G Y L T S F E M F N S T Y K L Y T H S Y L G F G L K A A	- - - - -	- - - - -	250
SEQ ID No-5	G T L D L G G A S T Q I T F L P Q F E K T L E Q T P R G Y L T S F E M F N S T Y K L Y T H S Y L G F G L K A A	- - - - -	- - - - -	250
CD39Human.seq	G A L D L G G A S T Q V T F V P Q - N Q T I E S P D N A - - L Q F R L Y G K D Y N V Y T H S F L L C Y G K D Q A A	R M E S E E L A D R V I L D V Y E R S L	- - - - -	261
SEQ ID No-3	- - R L A T L G A L E T E G	- - - - -	- T D G H T F R S A C L P R W L E A E W I F G G V K	287
SEQ ID No-5	- - R L A T L G A L E T E G	- - - - -	- T D G H T F R S A C L P R W L E A E W I F G G V K	287
CD39Human.seq	L W Q K L A K D I Q V A S N E I L R D P C F H P G Y K V V N V S D L Y K T P C T K R - F E M T L P F Q Q F -	R M E S E E L A D R V I L D V Y E R S L	- - - - -	314
SEQ ID No-3	Y Q Y G G N Q Q E G E V G F E P C Y A E V L R V V R G K	- - - - -	- L H Q P E E V Q R G S F Y A F S	330
SEQ ID No-5	Y Q Y G G N Q Q E G E V G F E P C Y A E V L R V V R G K	- - - - -	- L H Q P E E V Q R G S F Y A F S	330
CD39Human.seq	- - - E I Q G I G N Y Q Q C H Q S T I L E L F N T S Y C P Y S Q C A F N G I F L P P L Q G D F G A E S A F -	R M E S E E L A D R V I L D V Y E R S L	- - - - -	363
SEQ ID No-3	Y Y Y D R - - A V D T D M I D Y E K G G I L K V E D F E R K A R E V C D N L E N F T S G S P - F L - - - C M	- - - - -	- - - - -	378
SEQ ID No-5	Y Y Y D R - - A V D T D M I D Y E K G G I L K V E D F E R K A R E V C D N L E N F T S G S P - F L - - - C M	- - - - -	- - - - -	378
CD39Human.seq	- Y F V M K F L N L T S E K V S Q E K V T E M - M K K E C A Q P W E - - E I K T S Y A G V K E K Y L S E Y C F	R M E S E E L A D R V I L D V Y E R S L	- - - - -	414
SEQ ID No-3	D L S Y I T A L L K D G F G F A D S T	- - - - -	- - - - -	412
SEQ ID No-5	D L S Y I T A L L K D G F G F A D S T	- - - - -	- - - - -	402
CD39Human.seq	S G T Y I L S L L Q G Y H F T A D S W E H I H F I G K I Q G S D A G W T L G Y M L N L T	R M E S E E L A D R V I L D V Y E R S L	- - - - -	459
SEQ ID No-3	A L G A T F H L L Q S L G I S H	- - - - -	- - - - -	422
SEQ ID No-5	- - - - -	- - - - -	- V L R	405
CD39Human.seq	- - - - -	- - - - -	- - - - -	459

FIG 8

Figure 9

our ntpase	SEQ ID No-3	SEQ ID No-5	109
G S T G T R I H V Y T F V Q K T A G Q L P F L E G E I F D S V K P G L S A F V D Q P K Q G A E T V Q E L L E V	G S T G T R I H V Y T F V Q K M P G Q L P I L E G E V F D S V K P G L S A F V D Q P K Q G A E T V Q G L L E V	G S T G T R I H V Y T F V Q K M P G Q L P I L E G E V F D S V K P G L S A F V D Q P K Q G A E T V Q G L L E V	110
			110

ur ntpase	A K D S I P R S H W E R T P V V L K A T A G L R L L P E Q K A Q A L L L E V E E I F K N S P F L V P D G S V S	164
EQ III No-3	A K D S I P R S H W K K T P V V L K A T A G L R L L P E H K A K A L L F E V K E I F R K S P F L V P K G S V S	165
EQ III No-5	A K D S I P R S H W K K T P V V L K A T A G L R L L P E H K A K A L L F E V K E I F R K S P F L V P K G S V S	165

ur ntpase	IMDGSYEGILAWVTVNFLTGQLHGRGQETVGTLDLGCASTQITFLPQFEKTEL	219
ID No-3	IMDGSDEGILAWVTVNFLTGQLHGRQETVGTLDLGCASTQITFLPQFEKTEL	220
ID No-5	IMDGSDEGILAWVTVNFLTGQLHGRQETVGTLDLGCASTQITFLPQFEKTEL	221
ID No-7	IMDGSDEGILAWVTVNFLTGQLHGRQETVGTLDLGCASTQITFLPQFEKTEL	222

our ntpase	PRGYLTSEMFNSTE	KLYTHSYLGFGLKARLATA	GT DGH TFRSACLPR	274
PRGYLTSEMFNSTE	PRGYLTSEMFNSTE	KLYTHSYLGFGLKARLATA	GT DGH TFRSACLPR	275
PRGYLTSEMFNSTE	PRGYLTSEMFNSTE	KLYTHSYLGFGLKARLATA	GT DGH TFRSACLPR	276
PRGYLTSEMFNSTE	PRGYLTSEMFNSTE	KLYTHSYLGFGLKARLATA	GT DGH TFRSACLPR	277
PRGYLTSEMFNSTE	PRGYLTSEMFNSTE	KLYTHSYLGFGLKARLATA	GT DGH TFRSACLPR	278

W L E A E W I F C G V K Y Q Y G G N Q E G E M G F E P C Y A E V L R V V Q G K L H Q P E E V R G S A F Y A F S	329
W L E A E W I F C G V K Y Q Y G G N Q E G E V G F E P C Y A E V L R V V R G K L H Q P E E V Q R G S F Y A F S	330
W L E A E W I F C G V K Y Q Y G G N Q E G E V G F E P C Y A E V L R V V R G K L H Q P E E V Q R G S F Y A F S	331

ur ntpase	L L K D G L G F A E R H P I T - A H K E S E Q H R D W L G C H L S P A P V S G H H Q L R P S S T S E A C I	438
EQ ID No-3	L L K D G F G F A D S T V L Q L T K K V N N I E T G W - A L G A T F - - - - - H L L Q S L G I S - - - - -	427

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